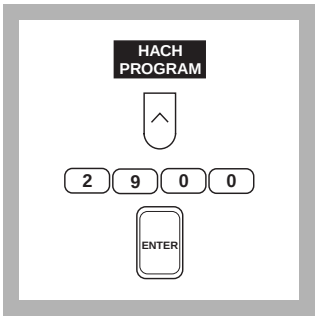
PHENOLS

✔ Method 8047 **4-Aminoantipyrine Method***

(0 to 0.200 mg/L)

Scope and Application: *For water, wastewater and seawater;*
*USEPA Accepted (distillation required)**. The estimated detection limit for program number 2900 is 0.001 mg/L phenol.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater*
** Procedure is equivalent to USEPA method 420.1 for wastewater.



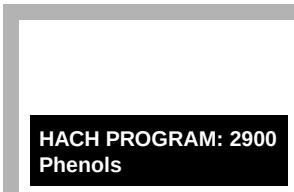
1. Press the soft key under ***HACH PROGRAM***.

Select the stored program number for phenols by pressing **2900** with the numeric keys.

Press: **ENTER**

Note: *Samples should be analyzed within four hours to avoid oxidation.*

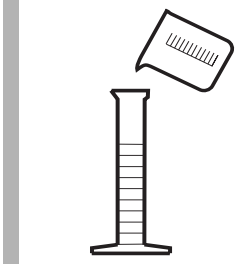
Note: *The Flow Cell and Sipper Modules cannot be used with this procedure.*



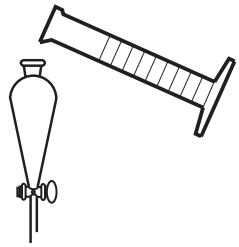
2. The display will show:

HACH PROGRAM: 2900 Phenols

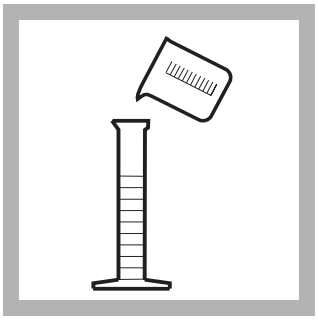
The wavelength (λ), **460 nm**, is automatically selected.



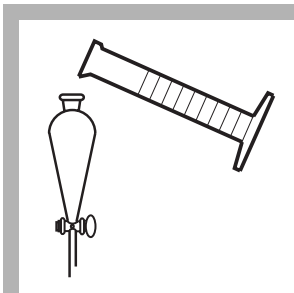
3. Measure 300 mL of deionized water in a 500-mL graduated cylinder.



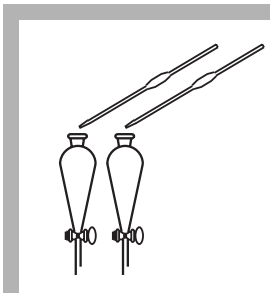
4. Pour the measured deionized water into a 500-mL separatory funnel (the blank).



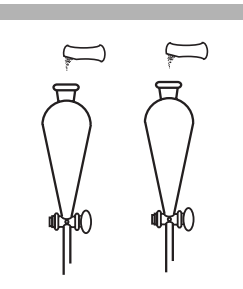
5. Measure 300 mL of sample in a 500-mL graduated cylinder.



6. Pour the sample into another 500-mL separatory funnel (the prepared sample).

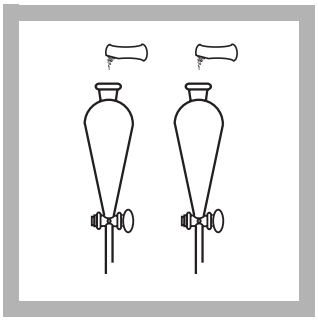


7. Add 5 mL of Hardness 1 Buffer to each separatory funnel. Stopper. Shake to mix.

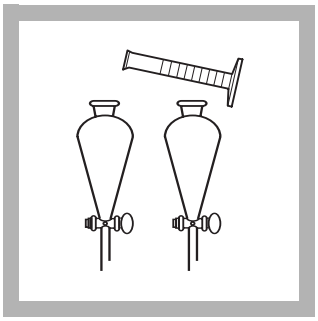


8. Add the contents of one Phenol Reagent Powder Pillow to each separatory funnel. Stopper. Shake to dissolve.

Note: *Spilled reagent affects test accuracy and is hazardous to skin and other materials.*

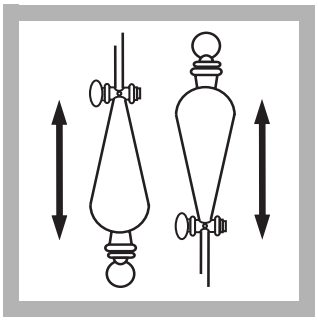


9. Add the contents of one Phenol 2 Reagent Powder Pillow to each separatory funnel. Stopper. Shake to dissolve.

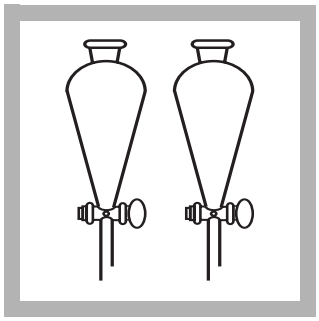


10. Add 30 mL of chloroform to each separatory funnel. Stopper each funnel.

Note: *Use chloroform only with proper ventilation.*

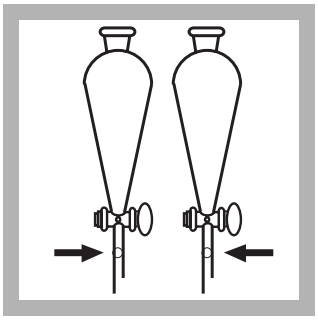


11. Invert each funnel and temporarily vent. Shake each funnel briefly and vent. Then vigorously shake each funnel for a total of 30 seconds (venting if necessary).

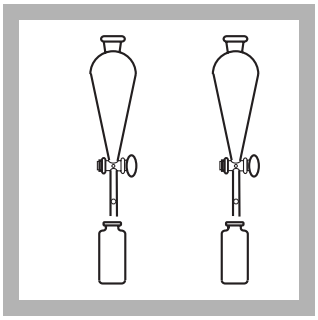


12. Remove the stoppers. Allow both funnels to stand until the chloroform settles to the bottom of the funnel.

Note: *The chloroform layer will be yellow to amber if phenol is present.*



13. Insert a large pea-sized cotton plug into the delivery tube of each funnel.

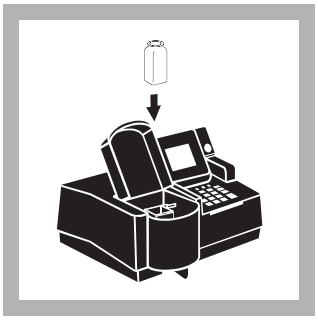


14. Drain the chloroform layers into separate sample cells (one for the blank, one for each sample).

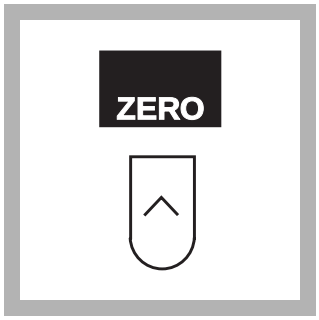
Note: *Filtering the chloroform layer through the cotton removes suspended water or particles. The volume of chloroform extract will be about 25 mL.*

Note: *Proceed promptly through the rest of the procedure since the chloroform will evaporate, causing high readings. Glass-stoppered sample cells are recommended.*

Note: *The water phase contains chloroform, which is a hazardous waste. See Pollution Prevention and Waste Management following these steps.*



15. Place the blank into the cell holder. Close the light shield.

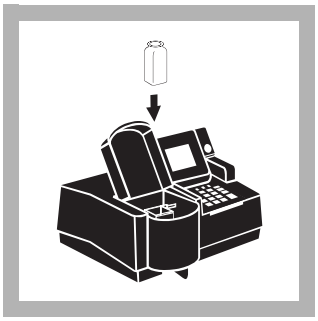


16. Press the soft key under ***ZERO.***

The display will show:

0.000 mg/L Phenol

Note: *For alternate concentration units press the soft key under* ***OPTIONS****. Then press the soft key under* ***UNITS*** *to scroll through the available options. Press* **ENTER** *to return to the read screen.*



17. Place the prepared sample into the cell holder. Close the light shield. Result in mg/L phenols (or chosen units) will be displayed.

Interferences

Table 1 Interfering Substances and Suggested Treatments

Interfering Substance	Interference Levels and Treatments
pH	The sample pH must be between 3 and 11.5 for best results.
Oxidizing or reducing agents	May interfere. Distill samples (see procedure below).
Sulfides or suspended matter	Distillation or the following pretreatment is necessary: **1.** Fill a clean 500-mL graduated cylinder with 350 mL of sample. Pour the sample into a clean 500-mL erlenmeyer flask. **2.** Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix. **3.** Filter 300 mL of the sample through a folded filter paper. Use this solution in Step 6.

Sample Collection, Storage and Preservation

Most reliable results are obtained when samples are analyzed within four hours after collection. Use the following storage instructions only if prompt analysis is not possible. Collect 500 mL of sample in clean glass containers and add the contents of two Copper Sulfate Powder Pillows. Adjust the pH to 4 or less with 10% Phosphoric Acid Solution. Store at 4 °C (39 °F) or lower and analyze within 24 hours.

Accuracy Check

Standard Solution Method
For greater accuracy, analyze standard solutions when new lots of reagent are first used.

1. Weigh out 1.00 g of Phenol, ACS. Transfer to a 1000-mL volumetric flask. Dilute to the mark with freshly boiled and cooled deionized water. This is a 1000-mg/L stock solution.

2. Pipet 10.0 mL of the 1000-mg/L stock solution to a 1000-mL volumetric flask. Dilute to the mark with deionized water. This is a 10-mg/L working solution.

3. Prepare a 0.200-mg/L standard solution by pipeting 10.0 mL of the working solution into a 500-mL volumetric flask. Dilute to the mark with deionized water.

4. Perform the phenol procedure described above using the prepared standard.

5. To adjust the calibration curve using the reading obtained with the standard solution, press the soft keys under ***OPTIONS, (MORE)*** then ***STD:OFF***. Press **ENTER** to accept the default concentration, the value of which will depend on the selected units. If an alternate concentration is used, enter the actual concentration and press **ENTER** to return to the read screen. See Section *1.5.5 Adjusting the Standard Curve* for more information.

Method Performance

Precision
Standard: 0.200 mg/L phenol

Program	95% Confidence Limits
2900	0.199-0.201 mg/L phenol

For more information on determining precision data and method detection limits, refer to Section *1.5*.

Estimated Detection Limit

Program	EDL
2900	0.001 mg/L phenol

For more information on derivation and use of Hach's estimated detection limit, see Section *1.5.2*. To determine a method detection limit (MDL) as defined by the 40 CFR part 136, Appendix B, see Section *1.5.1*.

Sensitivity
Program Number: 2900

Portion of Curve	ΔAbs	ΔConcentration
Entire Range	0.010	0.0020 mg/L

See Section *1.5.3 Sensitivity Explained* for more information.

Distillation

This procedure is in the *Hach Distillation Apparatus Manuals* in step-by-step illustrated format.

1. Set up the Hach Distillation Apparatus by assembling the general purpose apparatus as shown in the Distillation Apparatus Manual. Use the 500-mL erlenmeyer flask to collect the distillate. It may be necessary to use a laboratory jack to elevate the flask.
2. Place a stirring bar into the flask.
3. Measure 300 mL of water sample in a clean 500-mL graduated cylinder. Pour it into the distillation flask.
4. For proof of accuracy, use a 0.200-mg/L phenol standard (see *Accuracy Check*) in addition to the sample.
5. Using a serological pipet, add 1 mL of Methyl Orange Indicator to the distillation flask.
6. Turn on the stirrer power switch. Set the stir control to 5.
7. Add 10% Phosphoric Acid Solution drop-wise until the indicator changes from yellow to orange.
8. Add the contents of one Copper Sulfate Powder Pillow and allow to dissolve (omit this step if copper sulfate was used to preserve the sample). Cap the distillation flask.
9. Turn the water on and adjust it so a constant flow is maintained through the condenser. Set the heat control to 10.
10. Collect 275 mL of distillate in the Erlenmeyer flask, then turn the heat off.
11. Fill a 25-mL graduated cylinder to the 25-mL mark with deionized water. Add the water to the distillation flask.
12. Turn the still back on. Heat until another 25 mL of distillate is collected.
13. Using a clean graduated cylinder, re-measure the distillate to verify that 300 mL has been collected. The distillate is ready for analysis.

Calibration Standard Preparation

To perform a phenol calibration using the 4-aminoantipyrine method, prepare a 1000-mg/L phenol stock solution by adding 1.00 g Phenol, ACS to a 1000-mL volumetric flask. Dilute to the mark with freshly boiled and cooled deionized water. Prepare a 10.0-mg/L working solution by pipetting 10.0 mL of the 1000-mg/L stock solution into a 1000-mL volumetric flask using Class A glassware. Dilute to the mark with deionized water and mix thoroughly. Prepare standards containing 0.02, 0.08, 0.16, and 0.20 mg/L phenol as follows:

a. Into four different 500-mL volumetric flasks, pipet 1.0, 4.0, 8.0, and 10.0 mL of the 10 mg/L working solution using Class A glassware.
b. Dilute to the mark with deionized water. Mix thoroughly.

c. Using the 4-aminoantipyrine method and the calibration procedure described in the *User-Entered Programs* section of the *DR/4000 Spectrophotometer Instrument Manual*, generate a calibration curve from the standards prepared above.

Summary of Method

The 4-aminoantipyrine method measures all ortho- and meta- substituted phenols. These phenols react with 4-aminoantipyrine in the presence of potassium ferricyanide to form a colored antipyrine dye. The dye is then extracted from the aqueous phase with chloroform and the color is measured at 460 nm. The sensitivity of the method varies with the type of phenolic compound. Because water samples may contain various types of phenolic compounds, the test results are expressed as the equivalent concentration of phenol.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheet* for information specific to the reagents used. For additional information, refer to Section *1*.

Pollution Prevention and Waste Management

Phenol 2 Reagent Powder Pillows contain potassium ferricyanide. Both chloroform (D022) and cyanide (D001) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Chloroform solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal as a reactive waste. Be sure that cyanide solutions are stored in a caustic solution with a pH >11 to prevent release of hydrogen cyanide gas. See Section *1* for further information on proper disposal of these materials.

REQUIRED REAGENTS AND STANDARDS

	Cat. No
Phenols Reagent Set (100 tests)	22439-00
Includes: (3) 424-49, (2) 14458-17, (2) 1836-99, (2) 872-99	

Description	Quantity Required Per Test	Unit	Cat. No.
Chloroform, ACS	60 mL	4 L	14458-17
Hardness 1 Buffer Solution, pH 10.1	10 mL	500 mL	424-49
Phenol 2 Reagent Powder Pillows	2	100/pkg	1836-99
Phenol Reagent Powder Pillows	2	100/pkg	872-99
Water, deionized	300 mL	4 liters	272-56

REQUIRED EQUIPMENT AND SUPPLIES

Clippers, for opening powder pillows	1	each	968-00
Cotton Balls	1	100/pkg	2572-01
Cylinder, graduated, 50-mL	1	each	508-41
Cylinder, graduated, 500-mL	1	each	508-49
Funnel, separatory, 500-mL	2	each	520-49
Pipet, volumetric, Class A, 5.00-mL	1	each	14515-37
Ring, support, 4-inch	2	each	580-01
Sample Cells, glass-stoppered, 1-inch, matched pair	1	2/pkg	26126-02
Support, ring stand, 5 x 8 inch base	1	each	563-00

OPTIONAL REAGENTS AND STANDARDS

Copper Sulfate Powder Pillows	50/pkg	14818-66
Methyl Orange Indicator Solution, 0.5-g/L	100 mL MDB	148-32
Phenol, ACS	113 g	758-14
Phosphoric Acid Solution, 10%	100 mL MDB	14769-32
Sulfide Inhibitor Reagent Powder Pillows	100/pkg	2418-99

OPTIONAL EQUIPMENT AND SUPPLIES

Cylinder, graduated, 25-mL	each	508-40
Distillation Heater and Support Apparatus, 115 VAC	each	22744-00
Distillation Heater and Support Apparatus, 230 VAC	each	22744-02
Distillation Apparatus Set, general purpose	each	22653-00
DR/4000 Carousel Module Kit	each	48070-02
Filter Paper, folded, 12.5-cm	100/pkg	1894-57
Flask, Erlenmeyer, 500-mL	each	505-49
Flask, volumetric, Class A, 500-mL	each	14574-49
Flask, volumetric, Class A, 1000-mL	each	14574-53
Funnel, poly, 65-mm	each	1083-67
Jack, laboratory, 15 X 15 cm	each	22743-00
pH Paper, pH 1.0 to 11.0	5 rolls/pkg	391-33
Pipet Filler, safety bulb	each	14651-00
Pipet, serological, 1.0-mL	each	532-35
Pipet, volumetric, Class A, 1.00-mL	each	14515-35
Pipet, volumetric, Class A, 2.00-mL	each	14515-36
Pipet, volumetric, Class A, 3.00-mL	each	14515-03
Pipet, volumetric, Class A, 4.00-mL	each	14515-04
Pipet, volumetric, Class A, 10.00-mL	each	14515-38

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